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Effect of Storage at - 20 °C on the Concentration of Amino Acids in Plasma

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Summary: Amino acids were determined in whole and deproteinized plasma from six subjects, before and after storage at – 20 °C over a period of four weeks. When the native plasma was stored the following changes were observed after four weeks (fractions, mean ± SEM): increase of glutamic acid (to 1.39 ± 0.11), decrease of glutamine (to 0.91 ± 0.03), half-cystine (to 0.24 ± 0.09) and arginine (to 0.94 ± 0.02). In stored deproteinized plasma, only glutamine decreased slightly (to 0.96 ± 0.02) in four weeks. The concentration of 22 other amino acids remained unchanged irrespective of the type of storage.

It is concluded that amino acids are more stable in deproteinized than in native plasma during storage at - 20 °C for four weeks.

Einfluß der Lagerung bei - 20 °C auf die Aminosäurekonzentration im Plasma

Zusammenfassung: Im Plasma von 6 Patienten wurde die Konzentration von 23 Aminosäuren vor und nach Lagerung bei - 20 °C über 1–4 Wochen im Nativplasma und im enteiweißten Plasma bestimmt. Bei Lagerung des Nativplasmas kam es zu kontinuierlichen, zunehmenden Änderungen der Konzentrationen mehrerer Aminosäuren. Änderung nach vier Wochen (Fraktion der ursprünglichen Werte, $\bar{x}$ ± SEM): Anstieg der Glutaminsäure auf 1,39 ± 0,11, Abnahme von Glutamin auf 0,91 ± 0,03, Cystin auf 0,24 ± 0,09 und Arginin auf 0,93 ± 0,02. Bei Lagerung von enteiweißtem Plasma fiel lediglich die Glutamin-Konzentration gering auf 0,96 ± 0,02 ab, während sich die Konzentrationen der übrigen Aminosäuren nicht änderten.

Aus den vorliegenden Ergebnissen kann gefolgert werden, daß die Lagerung in Form eines enteiweißten Extraktes zur Bestimmung der Aminosäurenkonzentration von Plasma besser geeignet ist als in Form von Nativplasma.

Introduction

Stein & Moore (1) were the first to report, in 1954, that the amino acid concentration of whole plasma is influenced by storage even at - 20 °C. They observed a marked increase of glutamic acid and an extreme decrease of cystine by storage of whole plasma at - 20 °C for seven months. Similar observations on the instability of certain amino acids in plasma have since been repeatedly reported (2, 3, 4). However until now no systemic studies on the effect of storage on the amino acid concentration of whole plasma in comparison to deproteinized plasma have been published.

The purpose of the present study was to compare the effect of storage at - 20 °C for 1 to 4 weeks on the concentrations of 23 amino acids in whole plasma and deproteinized plasma.

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Material and Methods

Blood samples from six female patients with heart diseases (5 patients without liver diseases and one patient with liver cirrhosis) were drawn from the cubital vein in the fasting state at 8 a.m.. Each blood sample (8 ml) was put into plastic vials containing EDTA and immediately centrifuged at 2500 *g* for 10 minutes. One ml samples of native plasma were stored at - 20 °C for one and four weeks respectively and then rendered protein-free for amino acid analysis. Other samples of native plasma were deproteinized immediately with 30 g/l sulfosalicylic acid for amino acid determination. In addition 2 samples of 1 ml of the deproteinized plasma of each of the patients were stored for 1 or 4 weeks prior to the determination of the amino acids.

Plasma amino acid concentrations of each sample were measured twice by the ion-exchange column chromatographic method using a Beckman Amino Acid Auto Analyzer 119 CL. The internal standard used was α-amino guanidinopropionic acid. The calculations were performed by Beckman 126 Data System.

For determination of the change of the plasma amino acid concentrations after storage the mean value ± SEM of each amino acid is given as fraction of the initial value. For statistical analysis *Student*'s t test for paired data was used.

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Results

The changes in the concentration of each amino acid after storage for 1 or 4 weeks are presented in table 1. In the stored plasma, rendered protein-free immediately after sample collection, the amino acids proline and citrulline increased significantly after one week, whereas glutamic acid showed a significant increase after four weeks of storage. The concentration of the other amino acids remained essentially unchanged during four weeks with only glutamine showing a slight decrease.

In the stored plasma, rendered protein-free one week after sample collection, an increase of the glutamic acid was observed. Storage of deproteinized plasma samples for four weeks led to a significant decrease of arginine.

In stored samples of native plasma deproteinized after four weeks a marked increase of glutamic acid (to 1.39 ± 0.11) (mean ± SEM, initial values = 1.00) and an extreme decrease of half-cystine (to 0.24 ± 0.09) accompanied by only a slight decrease of glutamine (to 0.91 ± 0.03) and arginine (to 0.94 ± 0.02) were observed.

The concentration of the branched chain amino acids, valine, leucine and isoleucine and of the aromatic amino acids, phenylalanine and tyrosine remained unchanged during storage at - 20 °C for 4 weeks in native plasma as well as in deproteinized plasma.

Discussion

Since the first report of *Stein & Moore* (1) several observations have been published indicating that the concentration of certain amino acids may be influenced by storage even at low temperatures (2–6).

The concentrations of the majority of the amino acids determined in the present study remained constant in both samples of native plasma and in samples of deproteinized plasma when stored at - 20 °C up to 4 weeks. This also holds true for the branched chain amino acids and the aromatic amino acids the concentrations of which are changed in patients with severe liver disease (7). Consequently the molar ratio $\frac{\text{Val + Leu + Ile}}{\text{Phe + Tyr}}$, which has been assumed to be of importance in the development of chronic hepatic encephalopathy (8), did not change during storage.

An increase of the glutamic acid concentration in native plasma after storage has been reported by several investigators and this increase is apparently dependent on the temperature at which the samples are stored. When stored at + 4 °C (4) the rate fraction of increase was 0.45/day and when stored at - 15 °C (3) it was 0.29/3 days. By storage at - 68 °C the plasma glutamic acid concentration was found to increase 0.40 after one

Tab. 1. Changes of the amino acid concentration in plasma during storage at - 20 °C. The fractions of initial values (mean values ± SEM) are given (initial concentration = 1.00).

	Group A		Group B		Group C
Time of storage	1 week	4 weeks	1 week	4 weeks	4 weeks
Taurine	0.962 ± 0.036	0.991 ± 0.038	0.980 ± 0.046	1.022 ± 0.027	1.022 ± 0.023
Threonine	0.984 ± 0.005	0.993 ± 0.015	0.972 ± 0.013	0.991 ± 0.014	0.992 ± 0.015
Serine	1.021 ± 0.016	1.022 ± 0.009	0.996 ± 0.013	1.031 ± 0.035	1.015 ± 0.020
Glutamic acid	0.965 ± 0.043	0.923 ± 0.032	1.135 ± 0.065	1.118 ± 0.064	1.393 ± 0.111*
Glutamine	0.965 ± 0.020	0.957 ± 0.018*	0.943 ± 0.018*	0.933 ± 0.025*	0.912 ± 0.027*
Proline	1.082 ± 0.029*	1.063 ± 0.033	0.994 ± 0.037	1.085 ± 0.036	1.005 ± 0.021
Glycine	0.993 ± 0.011	0.997 ± 0.008	0.946 ± 0.025	1.007 ± 0.016	0.973 ± 0.016
Alanine	0.990 ± 0.016	1.041 ± 0.018	0.936 ± 0.035	0.986 ± 0.043	0.990 ± 0.017
Citrulline	1.045 ± 0.021*	1.053 ± 0.031	0.978 ± 0.059	0.993 ± 0.044	1.044 ± 0.028
α-Amino-n-butyric acid	1.012 ± 0.010	0.984 ± 0.030	1.017 ± 0.055	0.974 ± 0.044	0.957 ± 0.018
Valine	1.003 ± 0.009	1.012 ± 0.009	0.985 ± 0.015	1.018 ± 0.005	0.992 ± 0.012
half-Cystine	1.150 ± 0.110	1.137 ± 0.227	0.615 ± 0.087*	0.579 ± 0.060*	0.240 ± 0.093*
Methionine	1.032 ± 0.038	0.983 ± 0.023	0.997 ± 0.027	0.989 ± 0.033	0.948 ± 0.027
Isoleucine	0.989 ± 0.013	1.010 ± 0.005	0.979 ± 0.015	0.993 ± 0.017	0.996 ± 0.006
Leucine	1.017 ± 0.015	1.015 ± 0.007	1.011 ± 0.011	1.003 ± 0.015	0.995 ± 0.022
Tyrosine	1.001 ± 0.012	1.000 ± 0.008	0.985 ± 0.022	1.030 ± 0.022	1.018 ± 0.052
Phenylalanine	1.011 ± 0.007	1.000 ± 0.009	1.008 ± 0.012	0.999 ± 0.008	1.003 ± 0.005
Tryptophan	0.989 ± 0.020	0.999 ± 0.011	0.982 ± 0.011	0.984 ± 0.020	0.997 ± 0.013
Ornithine	1.011 ± 0.008	1.006 ± 0.009	0.990 ± 0.014	1.012 ± 0.013	1.014 ± 0.020
Lysine	1.032 ± 0.011	1.014 ± 0.012	1.012 ± 0.015	1.008 ± 0.013	1.006 ± 0.009
Histidine	1.016 ± 0.009	1.006 ± 0.006	0.978 ± 0.020	0.986 ± 0.023	0.984 ± 0.023
Arginine	1.018 ± 0.011	0.969 ± 0.010	0.983 ± 0.012	0.912 ± 0.011*	0.936 ± 0.020*
$\frac{\text{Val + Ile + Leu}}{\text{Tyr + Phe}}$	1.003 ± 0.006	1.015 ± 0.005	1.015 ± 0.007	1.033 ± 0.005	0.874 ± 0.031

* Significant difference when compared to initial values ($p < 0.05$)

A Storage of immediately deproteinized plasma samples

B Native plasma stored at - 20 °C for 1 week. After deproteinization, estimation of amino acids on the same day (left) and after 3 weeks (right).

C Storage of native plasma for 4 weeks.

week and to remain unchanged for several months thereafter (2). While *Dickinson* et al. (2) reported a 1.00 increase of glutamic acid after storage at - 20 °C for four weeks the change in the present study was distinctly less pronounced.

By storing deproteinized plasma at low temperature the concentration of glutamic acid was reported to increase slightly (2), moderately (3) or to remain stable (6). The findings in the present study are in accordance with the latter results.

The question whether the storage condition may influence the plasma glutamine concentration is still controversial. It has been reported that storage of native plasma at + 4 °C decreases the glutamine concentration at a rate of 0.20/day (4). When native plasma was stored at - 20 °C for eight months other authors observed a 0.50 decrease of the glutamine concentration whereas at - 68 °C the glutamine concentration remained unchanged (2). It has been assumed that the decreases of glutamine concentration might be due to the hydrolysis of glutamine to glutamic acid or to the fact that glutamine is cyclized to the ninhydrin-negative compound pyrrolidone carboxylic acid (2). In the present study the concentration of glutamine decreased slightly (to 0.91 ± 0.03) after storage at - 20 °C for four weeks.

The storage of deproteinized plasma at - 20 °C was reported to decrease the glutamine concentration at a rate of 0.002–0.0045/day (2, 3). The latter results are in accordance with our own findings. However in another report no changes of the glutamine concentration were obtained after storage of deproteinized plasma for four weeks at + 4 °C and - 70 °C (6).

The marked decrease of half-cystine concentration in native plasma obtained in the present study is in accordance with the data reported for the storage of plasma for several days at + 4 °C (4) and at - 15 °C (3). The fall of the concentration of half-cystine in native plasma following storage for one week was less pronounced than that observed by others under similar conditions (2).

In the present study tryptophan values remained stable stored at - 20 °C for 1–4 weeks in both whole plasma and deproteinized plasma. The decrease of the tryptophan concentration in native plasma observed by *Armstrong* et al. (3) and the decrease of 0.50 per month of the tryptophan value in stored deproteinized plasma reported by *Dickinson* et al. (2) remains to be explained.

In accordance with earlier reports (3, 5) we observed no substantial change of the methionine concentration in whole plasma or deproteinized plasma after storage for four weeks at - 20 °C. However it seems noteworthy that *Dickinson* et al. (2) reported that the methionine concentration in native plasma stored at - 20 °C decreased to 0.50 of the initial value within eight months although no significant change was seen after one month.

The plasma arginine concentration decreases rapidly at a rate of 0.61/day when whole blood is stored at + 4 °C (9). This change has been explained by the presence of arginase in erythrocytes (10). *Katz & Keck* (4) found an increase at a rate of 0.12/day of the arginine concentration in whole plasma stored at + 4 °C. In accordance with earlier reports (3, 5), when plasma is stored at - 20 °C the concentration of arginine remains nearly unchanged irrespective of whether or not the plasma is deproteinized before storage.

Dickinson et al. (2) observed an approximately 1.00 increase of the concentration of aspartic acid in both whole plasma and deproteinized plasma stored at - 20 °C. In accordance with *Katz & Keck* (4) who described a very low plasma concentration of aspartic acid, we also found only traces of this plasma amino acid in two of six patients. For this reason detailed values for aspartic acid are omitted from the present study.

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